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[A PRELIMINARY REPORT]

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In January, 1915, some diseased lettuce plants (*Lactuca sativa*) were sent to the United States Department of Agriculture from Nairn, La. The letter accompanying them stated that the disease was ruining the lettuce crop in that section, that about 200 acres of lettuce plants were badly infected, and that the fields looked as though a fire had swept through them.

At first the growers thought the disease was due to the excessive use of cottonseed meal, but it was reported in fields where no cottonseed meal was used. It occurred on high land, but was most prevalent on flat land. There had been excessive rainfall for three months in the affected region; however, there were fields within 10 feet of the infected area that showed no visible trace of infection.

The plants received by the Department were full-grown heads with some of the outer leaves entirely shriveled and dried and some of them in a soft-rotted condition. The centers of the heads were sound, but between the center and these dead outer leaves were others affected in varying degrees. In some places there were numerous separated spots with a water-soaked appearance. In other places the spots had fused. Portions of many leaves were in a bad condition, while other parts of the same leaves were sound.

Razor sections of areas showing the earliest evidence of the disease were examined under the microscope, and numerous bacteria were found in the cells and between them. Fungus threads were not detected. In the advanced stages of the disease the palisade cells and the loose parenchyma cells had collapsed. Some of the younger diseased areas were used for isolating the organism presumed to cause the trouble, the isolation being made by means of agar-poured plates. The organism so obtained was proved to be infectious.

Colonies appeared three days after pouring the plates. Those colonies which produced the disease when they were inoculated into healthy lettuce plants were later studied carefully on agar plates. When very young they are round with entire margins smooth, translucent, cream-white in reflected light, bluish in transmitted light, with fish-scale-like markings which are not always present and which do not seem to be on the surface. These markings disappear as the colonies get older. When 3 days old,

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many colonies have a denser margin which is lighter colored than the center. When older, the center is not always uniform in color. It may have yellowish bands or mottlings and patches of the lighter margin color in it. There is not always a definite light margin, and some colonies seem quite uniform throughout. The colonies range from 3 to 5 mm. in diameter. On agar stroke this mottling is present when the culture is young, but disappears in both stroke and plate colony as they get older. Inoculations have been made with the mottled colonies and those uniformly colored—that is, either cream-colored or bluish throughout. All types are infectious.

Using subcultures from single colonies, the disease was reproduced with this organism by needle-prick inoculations four different times (12 plants) and twice by spraying water suspensions of it on middle-aged plants growing in the greenhouses (7 plants). Checks held under the same condition of heat and moisture remained healthy. Reisolation inoculations by means of needle pricks were also made, and these, too, were successful.

The organism is a bacterium,[1] motile by means of from one to three polar flagella; it is non-gas-forming in peptone water with the sugars and alcohols tried (dextrose, lactose, saccharose, maltose, mannit, and glycerin). It did not cloud the closed end in any of the fermentation tubes, but it clouds beef bouillon +15 in less than 24 hours at 23° C. when transfers are made from beef bouillon. In 10 days the bouillon has become a lime-green color.[2] The organism clears sterile milk in 15 days without coagulation, the cleared fluid becoming a pale turtle-green color. It blues litmus milk and will grow in peptone-beef bouillon at temperatures ranging from 1.5° to 34.5° C., though it will not grow in bouillon at 36° C. The thermal death point lies between 48° and 49° C. It grows well in Uschinsky's and Fermi's solutions, changing them to pale Veronese green and water green in 3 to 5 days, but grows very faintly in Cohn's solution. The organism liquefies gelatin slowly at 18.5° C., one-half of the gelatin in test-tube cultures being liquefied in 10 days. On potato cylinders it produces a fleeting dark blue-green color. This striking color reaction develops promptly and disappears on the sixth day or earlier. It grows in bouillon over chloroform, tolerates malic, tartaric, and citric acid (0.1 to 0.2 per cent) added to neutral beef bouillon, but will not grow in neutral beef bouillon containing 0.3 per cent of these acids. It grows readily in neutral and in beef bouillon +5, moderately in −10 and −18, faintly in −20, but will not grow in −22 beef bouillon.

The organism withstands a limited amount of drying. A drop of a 1-day-old bouillon culture smeared over sterile cover glasses and kept in

[1] This use of the genus Bacterium is in accordance with the system of classification proposed by Erwin F. Smith *in his* Bacteria in Relation to Plant Diseases. v. 1, p. 171. Washington, D. C., 1905. (Carnegie Inst. Wash. Pub. 27.)

[2] Ridgway, Robert. Color Standards and Color Nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

the dark at room temperature (20° to 23° C.) will produce growth up to the eleventh day of drying when such covers are placed in tubes of beef bouillon.

It is not especially sensitive to sunlight. Petri dishes, one half covered with black paper and exposed bottom up to the noon-day sun in April on a sack of ice, developed 15 to 30 colonies on the uncovered parts exposed for 30 minutes, but none at 40 minutes. The covered part of the 30-minute plates developed from 130 to 150 colonies; that of the 40-minute plates developed from 30 to 55 colonies.

The organism likewise grows in neutral beef bouillon containing 3 and 4.5 per cent of sodium chlorid, but does not grow in the same medium with 5 per cent of common salt. It produces indol, but less abundantly than *Bacillus coli*, and does not reduce nitrates.

Stained from young agar cultures, the organism is a short rod with rounded ends. It is less than 1 to 1.25μ in diameter and 1.25 to 3μ long. It occurs singly, in pairs, and also in chains. Spores have not been observed. The organism stains readily with carbol fuchsin, gentian violet, methyl violet, and methylene blue. It is Gram-positive and is not acid-fast. The flagella were stained by Loeffler's flagella stain.

A bacterial disease of lettuce has been reported from the Vermont, the Massachusetts, the Florida, and the North Carolina experiment stations. Pietro Voglino (1904)[1] in Italy has reported a bacterial disease of lettuce and named his organism "*Bacillus lactucae.*" As the description of the organism reported in his paper does not agree with our own (pink, nonliquefying, spore-bearing, etc.), it is clear that the Louisiana organism is not the same as the Italian, but is possibly the same as some one of the unnamed forms previously isolated in this country and not carefully described. The name "*Bacterium viridilividum*, n. sp.," is suggested for the one under consideration, owing to its peculiar appearance on steamed potato.

For purposes of orientation, a short account of the literature on bacterial diseases of lettuce follows:

L. R. Jones (1893)[1] has given an account of a bacterial stem-rot of lettuce. A large bacillus was found in the diseased stems, but was not isolated. He reproduced the disease (1) by planting healthy plants in soil inoculated with fragments of lettuce plants affected by "stem rot," (2) by crushing a diseased lettuce head in a little water and pouring this water about the roots of healthy plants.

G. E. Stone (1907) mentions a bacterial disease of lettuce leaves which had been investigated by Mr. Percival C. Brooks six years earlier. It is stated that Mr. Brooks isolated an organism and produced positive results from inoculation experiments. As the disease was believed to

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 478.

be of little consequence, no extensive study was made. There is no description of his organism.

F. L. Stevens (1908), in a short report on a bacterial disease of lettuce, states that the bacteria isolated were rather long rod forms. His attempts at inoculation were unsuccessful.

H. S. Fawcett (1908) also reports a bacterial disease of lettuce. He likewise isolated an organism and reproduced the disease. His colonies on standard peptonized agar had indefinite margins and pearl-white foci. The organism stained readily in carbol fuchsin and aqueous gentian violet, but with difficulty in methylene blue.

O. F. Burger (1912) describes a bacterial disease of lettuce which he says is caused by a species of Pseudomonas. The disease begins at the center of the head, which blackens and then becomes soft. In the seed bed the disease appears as small black spots on the leaves. This does not seem to be the type of disease that occurred in Louisiana this year. Burger states that cultures of the bacteria were made and healthy lettuce plants were inoculated. In 10 days the inoculated plants were black and pulpy, while the checks were still healthy.

Bacterium viridilividum does not agree with the descriptions of any of the organisms mentioned by these writers. Voglino's (1904) organism evidently does not liquefy gelatin, and the colonies in lettuce gelatin change from an ivory-white color to a rosy tint. *B. viridilividum* liquefies gelatin, and is never ivory white or of a rosy tint. Fawcett's (1908) organism produces colonies with indefinite margins and pearl-white foci and stains with difficulty in methylene blue. *B. viridilividum* has definite margins, no pearl-white foci, and stains readily in methylene blue. Stevens's (1908) organism is a long rod form; *B. viridilividum* is a short rod. Stevens's inoculations were not successful. Jones (1893), Brooks (Stone, 1907), and Burger (1912) did not describe their organisms.

LITERATURE CITED

BURGER, O. F.
1912. Lettuce rot. Fla. Agr. Exp. Sta. Press Bul. 200, 2 p.

FAWCETT, H. S.
1908. Lettuce disease. *In* Fla. Agr. Exp. Sta. Rpt., [1907]/08, p. lxxx–lxxxvii, pl. 4–5.

JONES, L. R.
1893. A bacterial "stem-rot" of lettuce. *In* Vt. Agr. Exp. Sta. 6th Ann. Rpt., 1892, p. 87–88.

STEVENS, F. L.
1908. A serious lettuce disease. *In* N. C. Agr. Exp. Sta. 30th Ann. Rpt., [1906]/07, p. 72.

STONE, G. E.
1907. Bacterial disease of lettuce. *In* Mass. Agr. Exp. Sta. 19th Ann. Rpt., [1906], p. 163–164.

VOGLINO, Pietro.
1904. Sulla batteriosi delle lattughe. *In* Ann. R. Accad. Agr. Torino, v. 46, 1903, p. 25–33, 4 fig.